PB86-122603

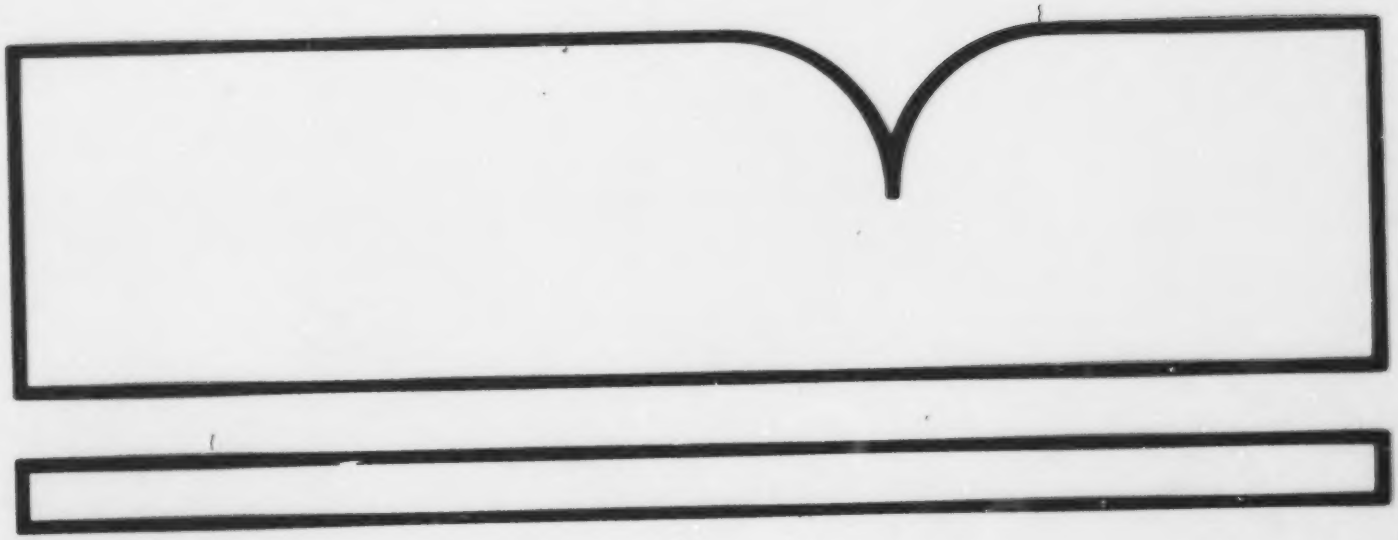
Thermophilic Anaerobic Biodegradation of Phenolics

Radian Corp., Austin, TX

Prepared for

Environmental Protection Agency
Research Triangle Park, NC

Oct 85



U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-122603

EPA/600/7-85/050
October 1985

THERMOPHILIC ANAEROBIC BIODEGRADATION OF PHENOLICS

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EPA Contract No. 68-02-3137

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/7-85/050		PB86 122603/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
Thermophilic Anaerobic Biodegradation of Phenolics	October 1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Frank J. Castaldi and Barbara J. Hayes	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Radian Corporation P. O. Box 9948 Austin, Texas 78766	11. CONTRACT/GRANT NO. 68-02-3137

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
EPA, Office of Research and Development Air and Energy Engineering Research Laboratory Research Triangle Park, NC 27711	Final; 6/84 - 6/85 14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES AEERL project officer is William J. Rhodes, Mail Drop 62B, 919/541-2853.

16. ABSTRACT The report gives results of a series of anaerobic microbial acclimation and treatment performance tests with synthetic phenolic substrates. The research is a feasibility level assessment of substituting anaerobic biodegradation of phenolics for solvent extraction. The tests showed the feasibility of biodegrading phenol and p-cresol to methane under thermophilic anaerobic conditions. The experimental data indicate that anaerobic biodegradation of phenolics under thermophilic conditions involves a dual system of bioaccumulation and biodegradation. Phenolics and unknown metabolites were accumulated in the anaerobic flocs of sludge, and these compounds were apparently slowly degraded over time. Despite periodic upsets, treatment improved as the phenolic loadings were incrementally increased during the experiments. This improvement indicates that, although acclimation of thermophilic anaerobic bacteria to phenolics is difficult, it can be accomplished using normal microbial cultivation techniques. Moreover, degradation intermediates identified during thermophilic anaerobic treatment of phenolics were similar to those found in effluents from anaerobic treatment systems that operate at lower temperatures.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Pollution · Waste Water	Pollution Control	13B
Phenols	Stationary Sources	07C
Biodeterioration	Thermophilic Anaerobic	06A
Anaerobic Processes	Biodegradation	06C
Thermophiles		
Coal Gasification		13H

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	69
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

NOTICE

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ABSTRACT

This report presents the results of a series of anaerobic microbial acclimation and treatment performance tests conducted with synthetic phenolic substrates. The research is a feasibility level assessment of substituting anaerobic biodegradation of phenolics for solvent extraction. The tests demonstrated the feasibility of biodegrading phenol and p-cresol to methane under thermophilic anaerobic conditions. The experimental data indicate that anaerobic biodegradation of phenolics under thermophilic conditions involve a dual system of bioaccumulation and biodegradation. Phenolics and unknown metabolites were accumulated in the anaerobic flocs of sludge, and these compounds were apparently slowly degraded over time. Despite periodic upsets, treatment improved as the phenolic loadings were incrementally increased over the course of the experiments. This indicates that although acclimation of thermophilic anaerobic bacteria to phenolics is a difficult process, it can be accomplished through normal microbial cultivation techniques. Moreover, degradation intermediates identified during thermophilic anaerobic treatment of phenolics were similar to those found in effluents from anaerobic treatment systems that operate at lower temperatures.

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SECTION 1

INTRODUCTION

Fixed-bed coal gasification wastewaters are generally high in biochemical oxygen demand. The largest fraction of the carbonaceous biochemical oxygen demand characteristic of these wastewaters can be attributed to the presence of phenolics. In addition to phenols, the wastewaters contain appreciable levels of cresols, xylenols, and aromatic heterocyclic compounds with nitrogen contained in the ring.

The chemistry of fixed-bed gasifier wastewaters offers the potential for a biological treatment alternative to solvent extraction pretreatment of high phenolic wastewaters. A conventional wastewater treatment system for fixed-bed coal gasification quench condensates consists of tar-oil separation, solvent extraction (phenolics removal), steam stripping (ammonia and acid gas removal), aerobic biodegradation, and assorted tertiary treatments (specific to discharge or reuse requirements). This system is energy intensive and presents both health and safety (worker exposure to solvents) and hazardous materials handling (phenolic by-product) considerations.

This research is a feasibility level assessment of substituting anaerobic biodegradation of phenolics for solvent extraction. This alternative treatment system would consist of tar-oil separation, steam stripping, anaerobic biodegradation, aerobic biodegradation, and assorted tertiary treatments. Anaerobic biodegradation would eliminate many of the health and safety concerns posed by solvent extraction treatment while also producing methane as a by-product. Methane may be generated in sufficient quantity to meet the power requirements of the entire alternative wastewater treatment train.

The anaerobic treatment system would provide simultaneous storage, equalization, and treatment of steam-stripped quench condensates and other process wastewaters. Since the process would receive wastewaters at temperatures in excess of 95°C, the anaerobic biodegradations should occur in the thermophilic temperature range (49 to 57°C) to minimize cooling requirements. These temperatures are consistent with equilibrium bioreactor temperatures estimated from heat balances on the anaerobic process. Operation in the thermophilic range would thus remove much of the cooling requirement typical of most mesophilic microbial treatment processes. (Traditionally, hot wastewaters are cooled by retention in large aerated impoundment ponds. This practice also permits the uncontrolled release of volatile compounds to the atmosphere.) A thermophilic anaerobic treatment process would restrict the release of volatile compounds because the emissions are controlled during treatment. The major gaseous emissions from the process are methane and carbon dioxide. The thermophilic temperature range should also optimize the rate of microbial conversion.

The purpose of this research was to study the feasibility of biodegrading phenolics to methane by thermophilic anaerobic treatment. The study was a feasibility level assessment which examined techniques for culturing thermophilic bacteria, acclimating these microorganisms to phenolics, and assessing toxicity thresholds for simple phenol. The study was conducted with bench-scale test reactors using synthetic substrates composed of phenol, p-cresol, organic acids, and basal salts. Both continuous-feed and batch treatment tests were conducted and treatment performance monitored. Where possible degradation intermediates were identified. These data provide the basis for assessing the feasibility of thermophilic anaerobic biodegradation of phenolics.

The report is organized into seven sections. Section 2 presents the conclusions of the research. Section 3 is a literature survey of past research into anaerobic treatment of aromatics. Section 4 presents the experimental approach and details the testing program as well as the analytical

quality control data. Section 5 presents the data analysis and discussion of results. Section 6 discusses technical questions not resolved by the research. References are listed in Section 7.

SECTION 2

CONCLUSIONS

The following conclusions result from the study of thermophilic anaerobic degradation of synthetic phenolic substrates:

- Experimental results indicate that phenol and p-cresol can be metabolized to methane under thermophilic anaerobic conditions. This microbial treatment process was sustained for a period of six months with continued improvement in treatment performance. A phenolic toxicity threshold for the microbial process was not evident when the degradations were performed with acclimated microorganisms. Relatively short acclimation periods were required to achieve consistent treatment performance (i.e., greater than 90 percent removal of phenol) at low substrate concentrations. However, extended periods of acclimation (i.e., greater than six hydraulic residence times) were required to achieve acceptable treatment at higher substrate (phenolic) loadings. Generally, acceptable treatment occurred coincident with the development of a flocculated biomass. However, the presence of dispersed microorganisms signalled poor treatability. These observations indicate that stable treatment performance could be maintained with conventional microbial acclimation techniques.

- The experimental data indicate that thermophilic anaerobic biodegradation of phenolics involves a dual system of bioaccumulation and biodegradation. Phenolics and unknown metabolites are accumulated in the anaerobic flocs and these compounds are released to the liquid at various times during treatment. Process control would specify a condition that optimizes the degradation of toxics in the liquid phase while maintaining an equivalent level of treatment for the sludges. Operating under this condition would serve to minimize the production of new cell mass.

- Although the test bioreactors experienced upset conditions in the early stages of the acclimation study, phenolic wastes were continuously fed to the systems throughout the upset periods. Despite these periodic upsets, system

performance improved as the waste loadings were incrementally increased over the course of the experiments. This indicates that although acclimation of thermophilic anaerobic bacteria to phenolics is a difficult process, it can be accomplished through normal microbial cultivation techniques.

- The experimental observations indicate that proportional increases in reactor biomass relative to pollutant loading will not guarantee that the individual phenolic compounds and intermediates will be uniformly degraded. This would imply that system kinetics may not fit a conventional microbial substrate utilization model.

- The experimental data indicate that degradation intermediates identified during thermophilic anaerobic treatment of phenolics were similar to those found in effluents from anaerobic treatment systems that operate at lower temperatures.

The following technical issues were not addressed in this study:

- The extent of accumulation of phenolics and breakdown metabolites on flocs of sludge and the kinetics of biodegradation for the absorbed species.

- The long-term impact on treatment performance due to the accumulation of phenolics on flocs of sludge in the anaerobic process, and whether this phenomenon presents a problem specific to thermophilic anaerobic biodegradation.

- The definition of key degradation intermediates and their impact on overall phenolic removal.

- The extent of metabolite production and the kinetics of biodegradation to methane for key degradation intermediates produced during thermophilic anaerobic treatment.

- The capacity of the thermophilic process to perform the anaerobic decomposition of a variety of methyl substituted phenols.

- The capacity of the thermophilic process to perform the anaerobic decomposition of the pollutant constituents in a wastewater matrix typical of most fixed-bed coal gasification quench condensates.

SECTION 3

ANAEROBIC DECOMPOSITION OF PHENOLICS

3.1 INTRODUCTION

Anaerobic treatment of wastewaters involves numerous biochemical and microbiological reactions resulting in an orderly and controlled dissimulation of a waste, without molecular oxygen acting as the electron acceptor. This microbial decomposition of complex organic substrates provides a source of energy and building materials to the bacterial cells which, in turn, convert a portion of the organic material to carbon dioxide and methane.

The conversion of complex organic waste to methane and carbon dioxide involves three stages or metabolic groups of bacteria - the fermentative, acetogenic, and methanogenic bacteria. The fermentative bacteria produce mainly short-chain organic acids, CO_2, and H_2. The acetogenic bacteria utilize the organic acids to produce acetate, CO_2, and H_2. The methanogens (methane formers) can metabolize only a few organic compounds and mainly obtain energy for growth from electrons generated during their oxidation of hydrogen.

The methanogenic bacteria are strict anaerobic microbes which are restricted in their specific substrate requirements. Only the lower normal fatty acids and the normal and isoalcohols containing fewer than five carbons and the gases H_2, CO, and CO_2 can be utilized as substrate by the methanogens (Bryant et al. 1967). Experimental observations of Bryant et al. (1967) indicated that the end products of the acetogenic stage of anaerobic processes which serve as substrate for the methanogenic bacteria consist primarily of acetate, H_2, CO_2, and methyl alcohol. Moreover, the major source of methane gas resulting from the fermentation of organic substances is attributed to the cleavage of acetate.

The group of facultative and anaerobic bacteria which is responsible for the acid fermentation stage, has a much faster rate of growth than the methanogenic bacteria responsible for the methane fermentation stage. As a result, the acid fermentation stage is relatively rapid so the methane fermentation stage is the rate-controlling step in the process. In particular, the acetate-utilizing methanogens have very slow rates of cell synthesis.

3.2 ANAEROBIC PHENOL DEGRADATION

The feasibility of employing mesophilic anaerobic fermentation for the treatment of high concentrations of long-chain organic and polycyclic nitrogen aromatic organic compounds has been demonstrated by a number of investigators (Parkin and Speece 1984; Wang et al. 1984). These studies have shown that when treating organics that are biodegradable, sufficient time must be provided for the microbes to develop the requisite biochemical machinery to accomplish bioconversion. Normally biostatic substances like phenolics present a more difficult acclimation problem because of their potential for chronic toxicity effects (i.e., low rates of substrate removal and biosynthesis). The acclimation of thermophilic anaerobes for the degradation of phenolics has not been previously reported; however, these microorganisms are believed to behave similarly to the mesophilic types.

A key step in the mesophilic anaerobic degradation of phenolics is scission of the aromatic ring. Working with methanogenic cultures developed from wastewater digestor sludge and from rumen contents, Keith (1973) observed conversion of benzoate to CO_2 and CH_4. Chmielowski et al. (1965, 1966) performed research on the anaerobic decomposition of phenol, p-cresol, and resorcinol. These studies were performed under batch fermentation conditions with 1000 mg/L of phenolic compounds (total of the monohydric and dihydric forms). The mixed bacterial populations gave complete conversion of phenolics to CH_4 and CO_2. According to Chmielowski et al. (1966) the initial stages of anaerobic degradation of phenols are characterized by the hydrogenation of the aromatic structure with the formation of some alicyclic compounds as

intermediates. The hydrogenation process was followed by ring-fission of alicyclic intermediates and then by methanogenesis of the degradation products (Chmielowski et al. 1966).

Fedorak and Hrudey (1984) performed screening experiments with four dimethylphenols, the three cresols, and phenol and found that only p-cresol and phenol are anaerobically degraded at 35°C with methane generation. Low concentrations of either degradable or nondegradable phenolics were not inhibitory to methanogenesis of other organics in the sludge. However, the phenol- and p-cresol-degrading acid-forming bacteria are susceptible to inhibition by the phenolic substrate.

Clark and Fina (1952) demonstrated the feasibility of degrading benzoic acid under mesophilic anaerobic conditions during methane fermentation. Healy and Young (1979) found that anaerobic microbial cultures acclimated to p-hydroxybenzoic acid were also capable of the degradation of benzoic acid and phenol without additional acclimation lag. This indicates that where there is a high degree of structural similarity among certain compounds, microbial acclimations can be accomplished with surrogate substrates. Investigation has indicated that the population of an anaerobic community that metabolizes substituted monoaromatic compounds (e.g., syringic acid or pyrogallol) are of two physiological types:

- those that remove methyl groups and
- those responsible for fermentative ring cleavage of hydroxylated benzoic acids and benzenes (Kaiser and Hanselmann, 1982).

Neufeld et al. (1980) evaluated the mesophilic anaerobic degradation of phenol using bioreactors operated in a batch-continuous mode. The end-products of a non-methanogenic anaerobic degradation of phenol were determined to be volatile organic acids and other organic (undefined) intermediates. The maximum specific rate of anaerobic phenol degradation was determined to be between 0.5 and 0.8% of the aerobic specific rate of

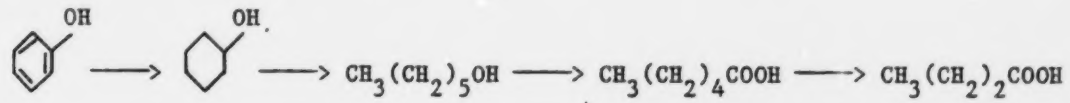
utilization. Khan et al. (1981) demonstrated the feasibility of biodegrading elevated concentrations of phenols in aqueous wastes by anaerobic treatment. Their study involved the application of fluidized-beds of granular activated carbon that provided media for attached anaerobic films. This experimental mesophilic anaerobic bioreactor was used to treat synthetic substrates of phenol and/or phenol and glucose mixtures in the presence of mineral salt solutions. Although adsorption accounted for some of the apparent system performance, phenols were degraded to methane gas in this process. The expanded-bed, granular activated carbon anaerobic filter was used to treat a 10 percent strength coal gasification wastewater generated from a fixed-bed process (Suidan et al. 1982). The feed wastewater was supplemented with a nutrient solution that included mineral salts, nitrogen, and phosphorus at levels required for biological growth. Treatment performance was satisfactory, although changing residual levels of phenol seemed to indicate a need for virgin carbon makeup to the system.

Studies conducted at Drexel University indicated that mesophilic anaerobic biodegradation of phenol, resorcinol, catechol, acetic acid, p-cresol, pyridine, benzoic acid, 4-methylcatechol, 3-ethylphenol, and hexanoic acid to methane gas is feasible. The degradation of these constituents was demonstrated by testing in serum bottles inoculated with both acclimated and unacclimated bacteria from an anaerobic sludge digester (Blum et al. 1984). However, elevated concentrations of these same constituents in inoculated serum bottles resulted in the inhibition of the conversion of acetate or propionate to methane. This effect was also noticed with the biodegradation of phenol.

Testing with continuously fed packed-bed anaerobic filters demonstrated the feasibility of anaerobically degrading phenol, resorcinol, catechol, p-cresol, and 3-ethylphenol to methane gas. In addition, o-cresol and 3,4 xylenol were also biodegraded; however, the degradations did not result in methane production (Blum et al. 1984). These tests were conducted with individual compounds using phenol enriched cultures of anaerobic bacteria.

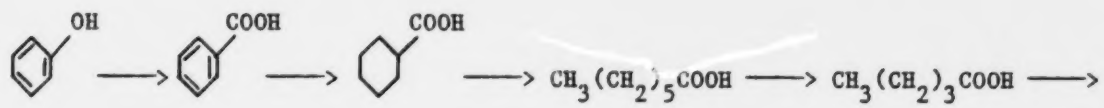
The combined degradation of phenol, resorcinol, and catechol was also demonstrated using anaerobic filters.

The possible mesophilic biochemical pathways for phenol degradation based on the works of Chmielowski et al. (1965, 1966), Keith (1973), Lawrence and McCarty (1969), and Williams and Evans (1973) can be described as follows:

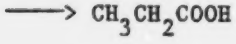


Phenol Cyclohexanol Hexanol Hexanoic acid Butyric acid

and



Phenol Benzoic acid 1-Cyclohexane carboxylate Heptanoic acid Valeric acid



Propionic acid

The biochemical conversion of phenol to either or both butyric and propionic acids is a strict fermentation which may involve several different microorganisms. The anaerobic oxidation of butyrate and propionate to acetate coincident with the reduction of carbon dioxide to methane can be described as follows (Smith 1980):

$$2CH_3CH_2CH_2COO^- + 4H_2O \longrightarrow 4CH_3COO^- + 4H_2$$

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$

$$2CH_3CH_2CH_2COO^- + 2H_2O + CO_2 \longrightarrow 4CH_3COO^- + CH_4$$

for butyric acid, and

$$4CH_3CH_2COO^- + 8H_2O \longrightarrow 4CH_3COO^- + 4CO_2 + 12H_2$$

$$12H_2 + 3CO_2 \longrightarrow 3CH_4 + 6H_2O$$

$$4CH_3CH_2COO^- + 2H_2O \longrightarrow 4CH_3COO^- + CO_2 + 3CH_4$$

for propionic acid. These biochemical conversions are mediated by acetogenic and methanogenic bacteria. It is generally assumed that certain species of methane-producing bacteria are capable of converting acetic acid to methane and carbon dioxide. However, all methane bacteria can obtain the energy required for growth by the anaerobic oxidation of molecular hydrogen.

To successfully maintain an anaerobic treatment process, the acid formers and the methane formers must be in a state of dynamic equilibrium. To establish and maintain that equilibrium, the reactor contents should be void of oxygen and free from inhibitory concentrations of certain metals, ammonia, and sulfur compounds. The heavy metals copper, zinc, and nickel are toxic at low concentrations to methane bacteria. In the presence of sulfides, however, some of these metals are precipitated. Ammonia is toxic at concentrations in excess of 3000 mg/L and inhibitory at levels greater than 1500 mg/L. This toxicity is related to pH which controls the amount of ammonia present in gaseous form. Free ammonia is more inhibitory than the ammonium ion. The acknowledged maximum concentration of soluble sulfide in an anaerobic system is 200 mg/L. The total sulfide concentration may be considerably higher. The loss of sulfide in the gas would permit higher concentrations of either sulfate or sulfide in the feed to the system provided the equilibrium concentration within the reactor is below the inhibitory level (Eckenfelder 1966; Switzenbaum 1983).

The methane forming bacteria function over a pH range of 6.5 to 7.5. When the rate of acid formation exceeds the rate of breakdown to methane, a process imbalance results in which the pH decreases, gas production falls off, and the carbon dioxide content of the gas increases. The control of pH is, therefore, essential to ensure a high rate of methane production.

The anaerobic process will effectively function over two temperature ranges, the high mesophilic range of 85 to 100°F (29-38°C) and the thermophilic range of 120 to 135°F (49-57°C). The overall rates of anaerobic substrate degradation are much higher in the thermophilic range, and this regime is considered advantageous for process operation given the elevated temperature of a steam stripped fixed-bed gasifier wastewater.

SECTION 4

EXPERIMENTAL APPROACH

4.1 INTRODUCTION

The anaerobic treatment feasibility study was designed as a first step in the determination of whether anaerobic microbial processes are applicable for the treatment of wastewaters from fixed-bed coal gasification. The primary objective of the study was to determine if phenols could be anaerobically degraded in a thermophilic temperature range with the coincident production of methane. Therefore, the major emphasis was placed on identifying the toxic limits of the anaerobic treatment process related to phenol loading.

This section describes the experimental approach used during the study to obtain data and reduce these data to meet the test objective. The remainder of this section presents:

- the description of the bench-scale anaerobic bioreactor,
- the description of the experimental test program, and
- the analytical quality control program.

4.2 BENCH-SCALE ANAEROBIC BIOREACTOR

The experimental anaerobic bioreactor test system used during the study is depicted in Figure 1. Two 10-liter and three 3.25-liter test systems were used during the study. Each test system consisted of a glass reaction vessel that was continuously fed and stirred to maintain completely-mixed conditions. Synthetic wastewaters were pumped into the reactor using a low speed peristaltic pump. Mixing was provided by a low speed high torque mixer.

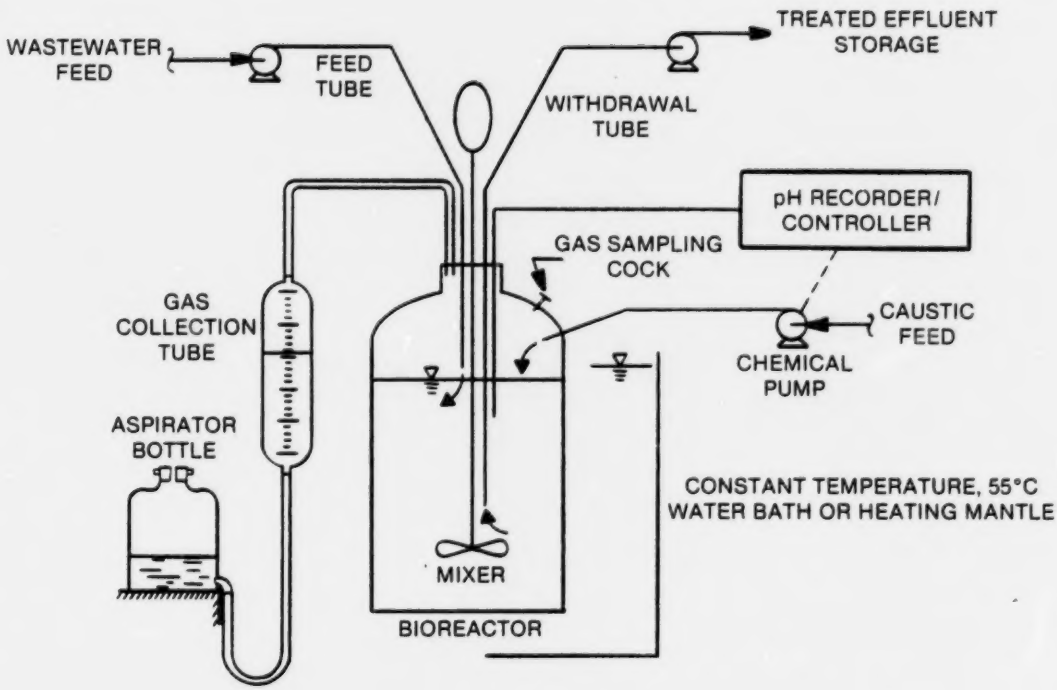


Figure 1. Anaerobic test bioreactor.

Heat to maintain the system in the desired thermophilic range of 55°C ±2°C was supplied by an electric heating mantle for the large units and a circulating water bath for the small units.

Initially, the large reactors were operated with high volume displacement of return sludges from a settling chamber which allowed effluent to be overflowed on a continuous basis. Due to the poor settling quality of the unacclimated microbial cultures, this arrangement allowed excessive loss of viable cells from the reactors. After five to six weeks, the settling chamber was removed from the system and the bioreactors were operated on a feed and draw basis.

4.3 TEST PROGRAM

The test program was divided into the following activities:

- Anaerobic seed acclimation with synthetic substrates and
- Performance testing using the acclimated seed cultures with synthetic substrates.

4.3.1 Seed Acclimation

Acclimation of the seed culture required the development of suitable anaerobic microorganisms for phenol degradation in the thermophilic temperature range. Initially, the two 10-liter bioreactors were used for this purpose and were seeded with anaerobic sludges from the Hornsby Bend municipal sludge treatment plant located in Austin, Texas. A 1:1 mixture of the reactor content and effluent of the Hornsby digestors was used as the seed culture. Prior to placement in the reactors, this material was strained to remove grit and any large extraneous particles. The straining process and the transfer of the seed to the bioreactors was performed while maintaining a protective nitrogen blanket over the sludge.

Feed to the reactors consisted of a synthetic waste mixture containing phenol, acetic acid, and propionic acid in a basal salts solution. After an initial acclimation period of about five to six weeks, p-cresol was added to the synthetic mixture. As the acclimation process progressed the phenol concentration was slowly increased in an incremental fashion.

During the acclimation period the reactors were monitored on a daily basis for flow, pH, and temperature and once per week for phenol, COD, and suspended solids. The analytical methods for these tests are discussed in Section 4.3.3. At the conclusion of 21 weeks of operation the sludges in the reactors were combined, after settling, in an effort to increase the total volatile suspended solids content of the bioreactors. At this time, these sludges were mixed with additional acclimated sludges obtained from a reactor seeded with biosludges from an activated sludge process treating a fixed-bed gasification wastewater. The combined sludges were also mixed with anaerobic municipal sludges to increase the population of the methanogenic microorganisms. This combined sludge reactor was then operated for an additional five to six weeks prior to dividing the microbial culture into three smaller reactors for the performance testing program.

4.3.2 Synthetic Wastewater Characteristics

Synthetic waste mixtures containing various concentrations of phenol, p-cresol, acetic acid, and propionic acid were prepared in a basal salts solution as the primary feed to the reactors. This mixture was selected to promote the cultivation of both the acetogenic and methanogenic groups of microorganisms. The formulation for the feed solution is given in Table 1. The solution was prepared in small batches of one to two liters which provided sufficient solution for two to four days feed. Phenol and sodium bicarbonate were weighed out in solid form using an analytical balance, while the other ingredients were added by pipeting concentrated or intermediate stock solutions. Contamination of the influent solutions by aerobic, phenol utilizing

TABLE 1. SEED ACCLIMATION SYNTHETIC WASTEWATER CHARACTERISTICS

Constituent	Substrate Solution[a] (mg/L)	Supplemental Nutrient-Buffer Solutions[b] A[c] (g/L)	B[c] (g/L)	C[d] (g/L)
Phenol	500 - 1000	--[e]	--	--
p-Cresol	50	--	--	--
Acetic Acid	250	--	--	--
Propionic Acid	250	--	--	--
$NaHCO_3$	2000	--	--	--
KH_2PO_4	--	18.0	--	--
NaCl	--	18.0	--	--
$CaCl_2 \cdot 2H_2O$	--	0.53	--	--
$MgCl_2 \cdot 6H_2O$	--	0.4	--	--
$MnCl_2 \cdot 4H_2O$	--	0.2	--	--
$CoCl_2 \cdot 6H_2O$	--	0.02	--	--
Na_2SO_3	--	1.0	--	--
$FeCl_2$	--	--	5.0	--
NH_4CO_3	--	--	--	165

[a] pH = 7.0

[b] After Neufeld et al. 1980.

[c] 5.5 ml of nutrient-buffer solution per liter of substrate solution.

[d] 5.0 ml of nutrient-buffer solution per liter of substrate solution.

[e] Dashes mean that constituent not added to solution.

microorganisms was minimized by preparing the solution in cooled, autoclave sterilized water.

4.3.3 Performance Testing Procedures

During performance testing the three 3.25-liter bioreactors were operated at the following conditions:

- Performance Reactor--Test #1 - Mixed Liquor Volatile Solids: 1400 mg/L
  Hydraulic Residence Time: 20 days
  Temperature: 55°C
  pH: 7.0 ± 0.2
  Phenol: 1000 mg/L
  p-Cresol: 50 mg/L
  Acetic Acid: 250 mg/L
  Propionic Acid: 250 mg/L

- Performance Reactor--Test #2 - Mixed Liquor Volatile Solids: 1600 mg/L
  Hydraulic Residence Time: 20 days
  Temperature: 55°C
  pH: 7.0 ± 0.2
  Phenol: 1200 mg/L
  p-Cresol: 50 mg/L
  Acetic Acid: 250 mg/L
  Propionic Acid: 250 mg/L

- Batch Reactor - Mixed Liquor Volatile Solids: 2000 mg/L
  Temperature: 55°C
  pH: 7.0 ± 0.2
  Phenol: shock loaded to 100 mg/L
  p-Cresol: shock loaded to 50 mg/L
  Acetic Acid: shock loaded to 50 mg/L
  Propionic Acid: shock loaded to 50 mg/L

The sampling and analysis schedule for the performance testing period is presented in Table 2. Chemical parameters of interest were analyzed by EPA-approved methods considering the possible interferences. The analytical methods used are listed in Table 3. Most analyses were initiated within one hour of sample collection. Samples for phenols and volatile organic acids analyses were filtered and stored at 4°C until analyses were performed during the last weeks of the program. A brief description of the methods used for phenols, volatile organics, and methane analyses follow in the next subsections.

4.3.4 Analytical Method for Phenols and p-cresol

Phenol and p-cresol were analyzed using an Antek 464 gas chromatograph (GC) with a 1.8m X 2mm stainless steel column packed with 0.1% SP1000 Carbopack C. Temperatures were: inlet at 180°C, column at 100°C with initial 3 minute hold and increased at 20°C per minute to a final temperature of 250°C, and the detector at 250°C. The carrier gas was nitrogen at 25 mL/min. A flame ionization detector (FID) was used with hydrogen and air flow rates to the detector at 30 mL/min and 300 mL/min, respectively. The signal from the detector was connected to a Hewlett Packard 3380 integrator. Samples for analysis were prepared by serially extracting a 5 mL sample volume with 1 mL of hexane at a pH greater than 10 followed by 1 mL of methylene chloride at a pH less than 2. A microliter of the methylene chloride extract was injected onto the column and was compared to the response of phenol and p-cresol standards prepared in methylene chloride. Typical phenol and p-cresol calibration curves are shown in Figure 2.

In addition to the GC method, the distillation/colorimetric method was also used during the study to determine influent phenolic levels. The GC method was optimized for the detection and quantification of p-cresol; therefore, the higher concentrations of phenols in the influent were biased low (about 20%) due to the instrument operating conditions. Use of the distillation/colorimetric method permitted an accurate assessment of influent phenolic levels.

TABLE 2. PERFORMANCE SAMPLING AND ANALYTICAL SCHEDULE

Analysis	Samples per Bioreactor			
	Type of Analysis	Influent	Effluent	Reactor
pH[a]	P[b]	X[c]	[d]	X
Chemical Oxygen demand	P	X	X	
Phenolics	P	X	X	
Total Organic Carbon	P		X	
Volatile Organic Acids	P	X	X	
Volatile Suspended Solids	C[e]			X
Gas Analysis[f]	C			X
Flow[g]	P	X		
Temperature[a]	P			X

[a]Continuous.

[b]P = Performance monitoring (3 to 5 samples per week).

[c]X = Samples taken for indicated analysis.

[d]Blank indicates no sample collected.

[e]C = Characterization monitoring (twice during study).

[f]Once during performance operation.

[g]Daily.

TABLE 3. SAMPLE ANALYSIS METHODOLOGY

Parameter	Method	Reference
pH	Electrometry	EPA 150.1
TOC	Oxidation NDIR (Beckman 915B)	EPA 415.1
COD	Dichromate Reflux/Titration	EPA 410
Phenol	Distillation/Colorimetry (4-AAP)	EPA 420.1
Phenolics, p-Cresol	Extraction/Gas Chromatography	SM 510 D
Volatile Organic Acids	Gas Chromatography	Sm
TSS/TVSS	Gravimetry 105°C/550°C	EPA 160.2; 160.4
Methane	Gas Chromatography	Le

EPA--U.S. EPA, Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, March 1979.

SM--APHA, AWWA, and WPCF, Standard Methods for the Examination of Water and Wastewater, 15th Edition, Washington, DC, American Public Health Association, 1980.

Le--Lentzen, D.E., D.E. Wagoner, E.D. Estes, and W.F. Guthenecht, "IERL-RTP Procedures Manual: Level I Environmental Assessments", 2nd Edition, EPA 600/7-78-201, January 1979.

Sm--Smith, P.H., Studies of Methanogenic Bacteria in Sludge, U.S. EPA 600/2-80-093, August 1980.

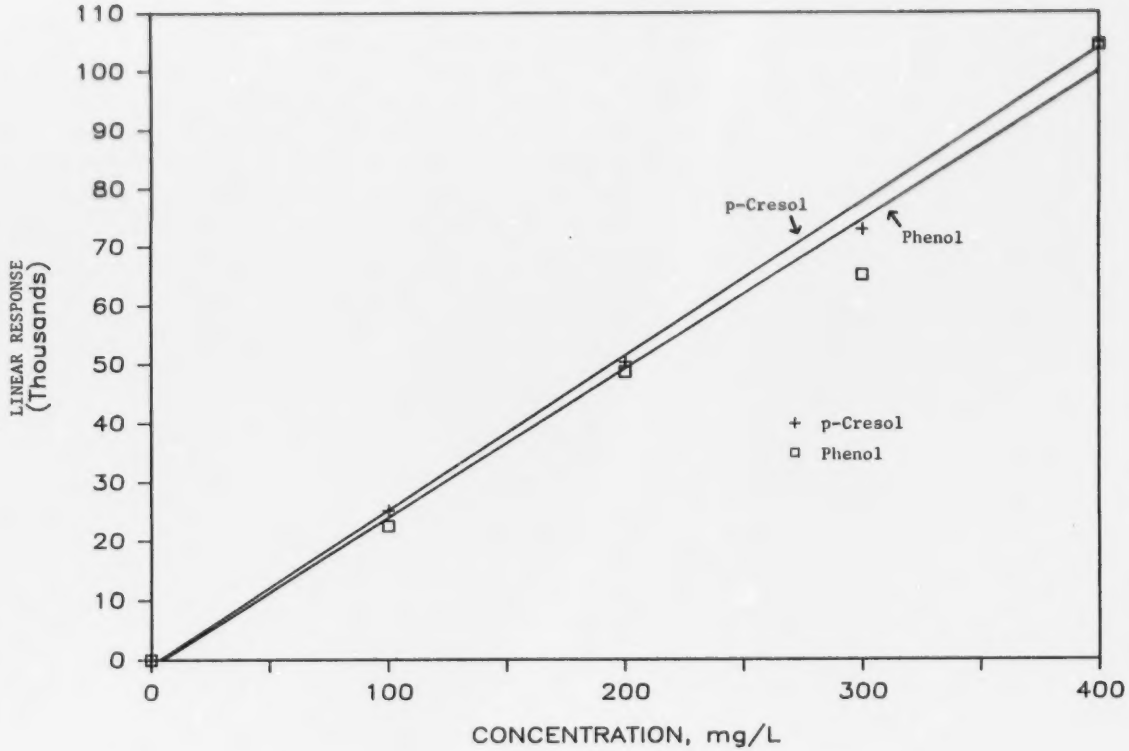


Figure 2. Typical phenol and p-cresol calibration curve.

4.3.5 Analytical Method for Volatile Organic Acids

Volatile organic acids including acetic and propionic acid were analyzed by gas chromatography using the following operating parameters:

Instrument: Antek 460 GC FID.

Column: 1.8m X 2mm stainless steel with 10% SP-1000 and 1% H_3PO_4 on 100/120 Chromosorb WAW.

Temp.: Injector at 150°C,
Column at 122°C, and
Detector at 150°C.

Gases: Nitrogen at 20 mL/min as the carrier,
Hydrogen at 30 mL/min, and
Air at 300 mL/min.

Samples were prepared by syringe filtering and acidifying with 30% H_3PO_4. Five microliters of sample were injected onto the column. Retention times and response factors were determined individually for the species of interest. Figure 3 shows typical calibration curves for acetic and propionic acid standards.

4.3.6 Analytical Method for Methane

Methane in the reactor gas was measured by gas chromatography using the following instrument parameters:

Instrument: Antek 460 GC FID.

Column: 1.8m X 2mm stainless steel with 60/80 mesh Porapak Q.

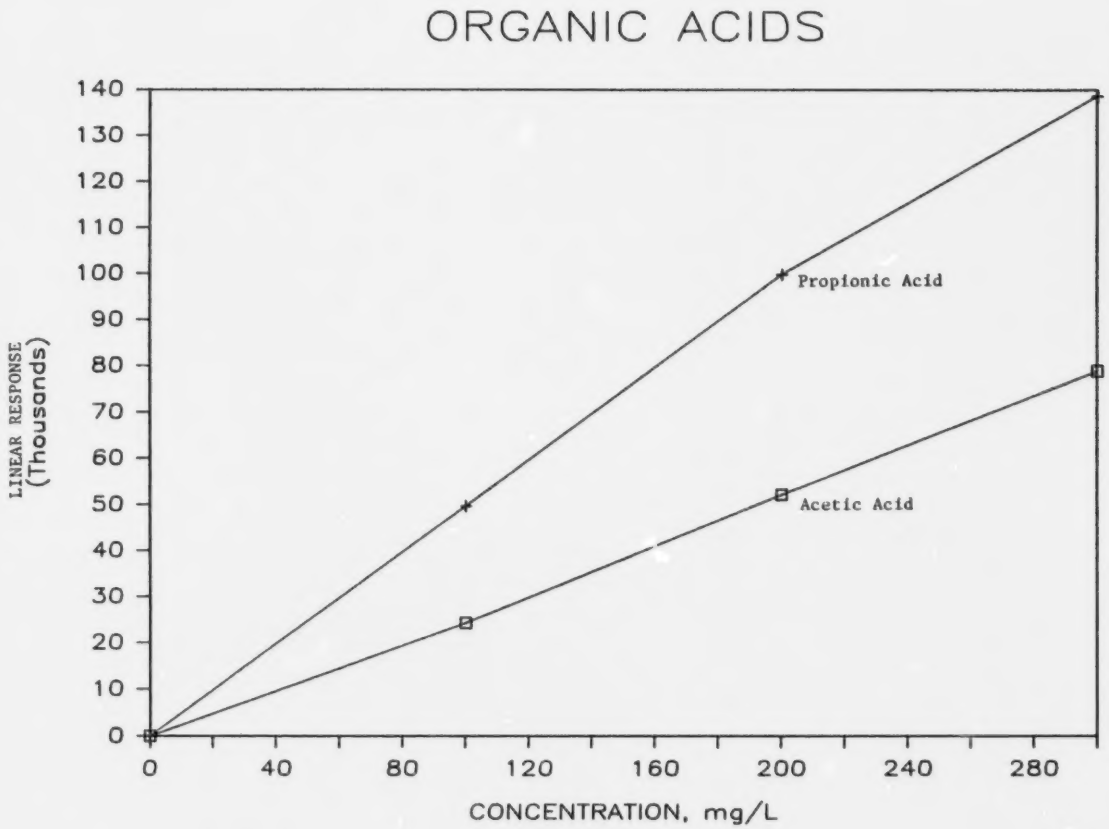


Figure 3. Typical organic acid calibration curve.

Gases: Nitrogen at 20 mL/min,
H_2 at 30 mL/min, and
Air at 300 mL/min.

Temp.: Column at 30°C and
Detector at 200°C.

Samples of the reactor gases were collected from the top of the reactors using a gas tight syringe inserted into a septum port at the top of the reactor. The gas was then injected into a sample loop in the GC and was compared with the retention times and response factors for certified gas standards.

4.4 ANALYTICAL QUALITY CONTROL

During the study, ten percent of the sample analyses were performed in duplicate or as replicate samples. A randomly selected sampling of the duplicate/replicate analyses is presented in Table 4 for the parameters COD, TOC, phenol, and volatile organic acids. The record of quality control data for each of the parameters is presented in the Appendix. In addition, EPA quality control samples were analyzed for COD and TOC during the course of the program. EPA samples were not available for most other parameters or the detection limit for the test was out of the range of the available standards. The results of the analysis of EPA samples are given in Table 5. These data indicate that satisfactory analytical quality control was maintained throughout the study.

TABLE 4. LABORATORY QUALITY CONTROL FOR RANDOMLY SELECTED DUPLICATE SAMPLES

Sample Identification	COD (mg/L)	Phenol (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)
I7-113084	3530	1170	290	360
	3530	1120	290	320
I7-120484	-[a]	1220	330	400
	-	1200	310	370
E7-121584	570	189	-	-
	550	193	-	-
E7-121984	1460	320	-	-
	1460	330	-	-
E7-122184	96	49	-	-
	108	45	-	-
E7-122884	495	-	-	-
	490	-	-	-
I6-121084	2600	-	230	230
	2290	-	220	200
E6-120984	560	230	-	-
	570	240	-	-
E6-113084	180	-	-	-
	160	-	-	-

[a]Dash means duplicate analyses not performed on sample for the parameter indicated.

TABLE 5. RESULTS OF THE ANALYSIS OF EPA QC SAMPLES FOR WATER QUALITY PARAMETERS

Parameter	EPA True Value (mg/L)	Radian Analyzed Value (mg/L)	% Recovery
TOC	92	100	109
COD #1 06-08-84	233	227	97
#2 01-10-85	233	215	90

SECTION 5

DATA ANALYSIS

5.1 INTRODUCTION

This section presents the results of a series of anaerobic microbial acclimation and treatment performance tests conducted with synthetic phenolic substrates. These tests were designed to demonstrate the feasibility of biodegrading phenolics under thermophilic anaerobic conditions. Two types of experiments were conducted:

- microbial seed acclimation tests using mixed cultures of anaerobic microorganisms grown under thermophilic conditions and
- treatment performance tests using acclimated microorganisms subjected to both continuous and batch biodegradation conditions.

The experimental results are discussed relative to the feasibility of achieving thermophilic anaerobic biodegradation of phenolics characteristic of most fixed-bed coal gasification wastewaters.

5.2 MICROBIAL SEED ACCLIMATION TESTING

Mesophilic anaerobes from a municipal sewage sludge digester were used to seed two test bioreactors. These sludges were fed a mixture of phenol and organic acids and were subjected to mixed liquor temperatures between 52 and 55°C. These two bioreactors were operated throughout the acclimation period at different substrate loadings. Although the bioreactors were loaded in a similar manner, they performed differently because of differences in the nature of the microbial cultures developed during seed acclimation. Later in

the experiment microorganisms obtained from an activated sludge process developed by the University of North Dakota (Gallagher 1984) to treat a dephenolated fixed-bed gas liquor were cultured under thermophilic anaerobic conditions. These sludges were used to supplement the active population of acclimated sewage sludge microorganisms in order to increase the relative diversity of the thermophilic microbial population.

The experimental data were obtained from three acclimation test conditions:

- thermophilic anaerobic biodegradation with flocculent microorganisms cultured from municipal sewage (Acclimation Test #1),
- thermophilic anaerobic biodegradation with dispersed microorganisms cultured from municipal sewage (Acclimation Test #2), and
- thermophilic anaerobic biodegradation with combinations of the two aforementioned microbial cultures inoculated with acclimated seed bacteria from an activated sludge process treating coal gasification wastewater (Acclimation Test #3).

Experimental data for the first test condition are presented in Table 6. The bioreactor was operated with an hydraulic residence time of 20 days and estimated mean cell residence times between 40 and 60 days. The influent phenol concentrations varied between 350 and 750 mg/L increasing throughout the duration of the test. The volatile organic acids, acetic and propionic, were fed to the bioreactor throughout the acclimation period in order to stimulate growth of methanogenic bacteria.

The microorganisms seemed to show an inherent ability for thermophilic anaerobic biodegradation of phenol early in the acclimation cycle. After three hydraulic residence times, a flocculent microbial mass developed that settled well and produced an effluent that was low in phenol. Overall chemical oxygen demand reduction lagged phenol removal and optimum COD removal was

TABLE 6. MICROBIAL SEED ACCLIMATION TEST #1

Test Day	Influent			Effluent		Reactor			
	Avg. pH	Phenol (mg/L)	COD (mg/L)	Phenol (mg/L)	COD (mg/L)	Avg. Temp. (°C)	Avg. pH	TSS (mg/L)	TVSS (mg/L)
7	-[a]	470	1770	63	650	52.8	7.8	1520	1020
13	7.4	530	1850	186	920	53.3	7.1	1560	1120
20	7.8	460	1650	200	880	52.8	7.3	1100	770
26	7.9	500	1230	140	500	53.3	7.1	960	710
33	7.8	920[b]	3670	315	960	54.4	6.9	980	730
40	7.5	450	1200	322	1090	54.4	7	870	700
46	8.1	490	1560	244	880	53.9	7.2	830	670
54	7.7	530	2050	140	510	-	-	3020[c]	1920[c]
59	-	470	1790	68	250	-	-	-	-
61	7.8	550	1960	67	380	52.2	7.1	2340	1600
63	-	520	2050	80	580	-	-	-	-
66	-	480	1960	137	530	-	-	-	-
68	7.6	480	1980	85	480	51.7	7.2	2020	1440
70	-	390	2280	121	510	-	-	1800	-
73	-	460	1730	130	490	-	-	-	-
75	7.4	390	1630	105	400	51.7	7.2	1800	1240
77	-	350	1680	71	345	-	-	-	-
80	-	510	2180	87	510	-	-	-	-
82	7.4	520	1450	115	620	52.8	7.1	1800	1220
84	-	490	2010	114	580	-	-	-	-
87	-	220	1130	69	410	-	-	-	-
89	7.7	520	2540	50	-	53.9	7.1	1690	1210
91	-	500	2220	48	400	-	-	-	-
93	-	460	1510	72	514	-	-	-	-
96	6.7	510	2080	81	270	53.9	6.7	1710	1080
98	-	460	2150	60	250	-	-	-	-
101	-	470	1850	50	300	-	-	-	-
103	7	515	2120	46	260	53.3	7	1940	1260
105	-	478	1980	20	160	-	-	-	-
110	7	488	1540	5	41	53.3	7	1840	-
112	-	507	1540	4.8	290	-	-	-	-
115	-	495	2040	7.6	313	-	-	-	-
117	7.2	518	2160	3.7	128	53.3	7.2	1370	750

(continued)

TABLE 6. (continued)

Test Day	Influent			Effluent		Reactor			
	Avg. pH	Phenol (mg/L)	COD (mg/L)	Phenol (mg/L)	COD (mg/L)	Avg. Temp. (°C)	Avg. pH	TSS (mg/L)	TVSS (mg/L)
119	-	518	2030	3.9	125	-	-	-	-
122	-	735	2860	12	137	-	-	-	-
124	7	733	-	26	196	52.8	7.1	1210	890
126	-	740	2670	46	114	-	-	-	-
129	-	708	1623	33	153	-	-	-	-
131	6.8	-	1560	3.5	95	52.8	7.2	1200	900
133	-	752	2480	4.8	81	-	-	-	-
136	-	742	2180	5.4	73	-	-	-	-
138	7.4	677	2390	6.8	92	53.3	7.1	1010	810
140	-	645	1750	5.8	100	-	-	-	-
143	-	685	2660	6.2	82	-	-	-	-
145	6.9	724	2070	49	220	53.3	6.9	1050	900

[a]Blank means parameter not analyzed.
[b]Transient increase in waste load (i.e., feed to reactor).
[c]Reactor reseeded to increase population of methane bacteria.

not obtained until after approximately six hydraulic residence times. Methane was detected in the off gas from the bioreactor whenever these gases were sampled during the acclimation period.

The bioreactor was reseeded with municipal sewage sludge during the acclimation test period. This occurred after two-and-one-half hydraulic residence times of operation. The reseeding was initiated to increase the resident methane bacteria population in the bioreactor because it was felt that the development of these microorganisms restricted system performance. Although the bioreactor consistently reduced the phenol concentration in the wastewater, the apparent level of methane produced did not keep pace with the rate of phenol removal during the early stages of the acclimation. However, this was not unexpected because methane forming bacteria are usually slow to develop in most anaerobic treatment processes that treat industrial effluents (Switzenbaum, 1983). The bioreactor also maintained relatively low concentrations of volatile cell mass throughout the test and the apparent rate of growth of new biosludge was negligible.

Experimental data for the dispersed microorganism test bioreactor (Acclimation Test #2) are presented in Table 7. The bioreactor was operated with an hydraulic residence time of 20 days and estimated mean cell residence times of less than 40 days. The influent phenol concentrations varied between 350 and 500 mg/L increasing throughout the duration of the test. Acetic and propionic acids were fed to the bioreactor during the acclimation period in order to stimulate the growth of methanogenic bacteria.

The thermophilic anaerobic biodegradation of phenol was relatively poor throughout the testing period with this bioreactor. System treatment performance for phenol removal did improve after approximately six hydraulic residence times. This increased phenol removal was coincident with effective COD reduction across the reactor and the subsequent occurrence of bioflocculation. The poor treatment performance associated with this acclimation test reactor is probably due to the loss of viable cell mass during treatment which is

TABLE 7. MICROBIAL SEED ACCLIMATION TEST #2

Test Day	Influent			Effluent		Reactor			
	Avg. pH	Phenol (mg/L)	COD (mg/L)	Phenol (mg/L)	COD (mg/L)	Avg. Temp. (°C)	Avg. pH	TSS (mg/L)	TVSS (mg/L)
7	-[a]	470	1770	33	500	52.2	7.7	1660	1100
13	7.4	530	1850	170	830	52.2	7.1	1600	1060
20	7.8	460	1650	195	940	53.9	7.1	1630	1130
26	7.9	500	1230	80	350	53.9	7.1	1200	920
33	7.8	920[b]	670	320	990	53.9	7.1	1200	920
40	7.5	450	1200	332	1040	53.9	7	920	720
46	8.1	490	1560	285	960	53.9	7	930	730
54	7.7	530	2050	270	1200	52.2	6.8	2460[c]	1630[c]
59	-	470	1790	176	620	-	-	-	-
61	7.8	550	1960	265	900	51.7	6.8	2520	1700
63	-	520	2050	190	796	-	-	-	-
66	-	480	1960	160	440	-	-	-	-
68	7.6	480	1980	320	950	51.7	6.9	2220	1460
70	-	390	2280	290	1000	-	-	1840	-
73	-	460	1730	310	940	-	-	-	-
75	7.4	390	1630	260	790	-	-	1680	1100
77	-	350	1680	250	700	-	-	-	-
80	-	510	2180	236	720	-	-	-	-
82	7.4	520	1450	194	740	52.8	6.7	2080	1380
84	-	490	2010	164	890	-	-	-	-
87	-	220	1130	140	660	-	-	-	-
89	7.7	520	2540	140	400	53.9	6.9	1150	770
91	-	500	2220	138	540	-	-	-	-
93	-	460	1510	178	800	-	-	-	-
96	6.7	510	2080	168	520	55.0	6.9	1500	1060
98	-	460	2150	180	600	-	-	-	-
101	-	470	1850	160	560	-	-	-	-
103	7	515	2120	176	610	54.4	6.9	1720	1160
105	-	478	1980	173	540	-	-	-	-
110	7	488	1540	211	510	53.9	6.8	1560	-
112	-	507	1540	204	580	-	-	-	-
115	-	495	2040	174	710	-	-	-	-
117	7.2	518	2160	192	670	52.8	6.9	1240	750
119	-	518	2030	200	630	-	-	-	-
122	-	524	2000	176	500	-	-	-	-
124	7	484	1730	152	462	52.8	6.9	1050	-
126	-	493	2160	152	438	-	-	-	-
129	-	393	-	98	310	-	-	-	-
131	6.8	-	1330	100	315	52.8	7.1	1020	770
133	-	456	1970	52	195	-	-	-	-
136	-	480	1500	18	81	-	-	-	-
138	7.4	513	1850	4.4	76	53.3	7.1	850	730
140	-	403	1170	2.2	58	-	-	-	-
143	-	484	1980	21	214	-	-	-	--
145	6.9	468	1060	61	210	53.3	7.1	750	670

[a] Blank means parameter not analyzed.
[b] Transient increase in waste load (i.e., feed to reactor).
[c] Reactor reseeded to increase population of methane bacteria.

characteristic of a dispersed growth system. Once the biosludge began to flocculate, treatment performance improved measurably. The reason for the lack of bioflocculation in the early stages of acclimation testing is not known. However, after flocculent microorganisms developed in the test reactor the treatment performance was comparable with the previous test system.

The dispersed microorganism bioreactor was also reseeded during the acclimation test period. However, this acclimation reactor still maintained relatively low concentrations of volatile cell mass throughout the test as was the case for Acclimation Test #1. The biosynthesis of new cell mass seems to be limiting in both test systems.

Gas volume was not measured during the study because the test reactors were not gas tight. The test equipment used in the experiment did not permit the installation of an effective seal at the point where the mixer shaft entered the reactor. However, gas samples were collected from the top of the reactor at various times during the acclimation study and these samples were analyzed for the presence of methane. These data are presented in Table 8 for both test conditions. Generally, there did not exist a relationship between percent removal of phenol and the quantity of methane detected in the reactor gas. Nevertheless, the data demonstrate that methane was produced during thermophilic anaerobic degradation of phenol, and the system has the potential for a favorable conversion of wastewater COD to a useful energy by-product.

Both acclimation reactors experienced upset conditions in the early portion of the study. However, phenolic wastes were still fed to the test systems throughout the upset period and system performance improved as the waste loadings were incrementally increased over the course of the experiment. This indicates that although acclimation of thermophilic anaerobic bacteria to phenolics is a difficult process, it can still be accomplished within a reasonable time period through normal microbial cultivation techniques.

TABLE 8. METHANE PRESENT IN GAS FROM ACCLIMATION BIOREACTORS

	Test #1		Test #2	
Test Day	Methane ppmv[a]	Phenol Removal %	Methane ppmv	Phenol Removal %
20	25	56	740	58
26	2900	72	4500	88
33	10	66	4	65
40	12	28	10	36
46	16	50	25	42
54	ND[b]	73	580	47
68	396	82	2170	33
88	1845	90	130	73
109	265	99	150	57

[a] ppmv = parts per million by volume.

[b] ND = not detected.

Experimental data for the third seed acclimation test condition are presented in Table 9. The biosludges examined in this test were comprised of the acclimated sewage sludges developed in the previous tests and additional seed bacteria from an activated sludge process treating coal gasification wastewater (University of North Dakota).

This acclimation bioreactor was operated with an hydraulic residence time of 20 days and a mean cell residence time of approximately 60 days. Influent phenol concentrations were increased to 1000 mg/L. In addition to simple phenol, the system was fed 50 mg/L of p-cresol, 250 mg/L of acetic acid, and 250 mg/L of propionic acid. These same substrates would be used later during performance testing.

Test data at this higher phenol loading indicate a continued increase in treatment performance with duration of the acclimation period. No apparent substrate inhibition effects are evident from these data, and the potential exists for higher phenol loadings with each increase in volatile cell mass in the reactor.

5.3 PERFORMANCE TESTING

5.3.1 Continuous-feed Biodegradation

Performance testing consisted of two continuous-feed biodegradation experiments conducted with similar populations of acclimated thermophilic anaerobic bacteria. Each experiment was designed to track the change in phenol, p-cresol, acetic acid, and propionic acid in a test reactor. Treatment performance data for the first test are presented in Table 10. This reactor was fed phenol at 1000 mg/L, p-cresol at 50 mg/L, and volatile organic acid as equal quantities of acetic and propionic acids (i.e., 200 to 400 mg/L). The bioreactor was operated with an hydraulic residence time of 20 days and a mean cell residence time of approximately 60 days. The system

TABLE 9. MICROBIAL SEED ACCLIMATION TEST #3

Test Day	Influent		Effluent		Reactor			
	Phenol (mg/L)	COD (mg/L)	Phenol (mg/L)	COD (mg/L)	Avg. Temp. (°C)	Avg. pH	TSS (mg/L)	TVSS (mg/L)
1	-[a]	-	49	210	-	6.7	2660	1880
2	500	1890	50	200	51.7	6.9	-	-
5	740	2060	84	440	-	-	-	-
9	930	2880	100	410	52.8	6.9	-	-
12	1180	2680	110	500	-	-	-	-
14	930	3140	120	570	-	-	2220	-
16	1070	3150	150	550	52.2	6.9	-	-
19	930	2830	150	550	-	-	-	-
27	1150	2980	240	620	53.9	7	-	-
29	920	2800	200	540	-	-	-	-
31	880	2880	120	490	-	-	1950	-
33	980	2600	74	380	53.9	6.9	-	-
36	1160	3160	16	240	-	-	-	-
38	860	3410	24	250	-	-	1840	1270
40	870	2930	34	350	54.4	6.9	-	-
43	990	3200	44	370	-	-	-	-
45	1060	3310	61	300	-	-	-	-
50	1100	2730	110	420	53.9	6.95	1540	1100

[a]Blank means parameter not analyzed.

TABLE 10. PERFORMANCE TEST #1

Test Day	Influent					Effluent						Reactor[a]	
	Phenol (mg/L)	Cresol (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	COD (mg/L)	Phenol (mg/L)	Cresol (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	COD (mg/L)	TOC (mg/L)	Temp (°C)	pH
1	-[b]	50	300	280	3040	<5	<5	<5	97	170	57	53	7.15
4	-	-	370	310	3100	-	-	<5	-	-	-	-	-
5	1030	31	-	-	2970	<5	<5	<5	96	231	88	55	7.2
6	-	-	-	-	3120	79	<5	<5	-	234	101	54	7.2
7	-	-	-	-	-	<5	<5	<5	94	124	-	54	7.1
8	1070	38	350	440	-	-	-	<5	-	-	-	53	7.15
10	-	-	-	-	-	230	<5	<5	103	570	178	-	-
11	970	48	230	230	2440	<5	<5	<5	110	190	-	53	7.15
12	-	-	320	490	-	55	<5	<5	86	115	93	53	-
13	-	-	310	240	3140	10	<5	<5	110	150	-	53	7.2
14	-	-	170	160	-	6.2	<5	-	-	126	-	52.5	7.2
15	-	-	97	115	2890	<5	<5	<5	88	134	-	53	7.2
16	-	-	-	-	-	<5	<5	<5	108	153	-	53	7.2
17	-	-	-	-	-	75	<5	36	95	282	-	53	7.2
18	1000	47	250	300	2570	190	-	-	-	-	-	53	7.2
19	-	-	-	-	-	120	<5	84	16	350	129	53	7.2
20	1000	48	200	590	2960	<5	<5	6	90	210	-	53	7.15
22	-	14	<5	<5	2680	16	<5	<5	100	204	139	53	7.2
25	1020	31	-	95		<5	<5	<5	80	102	-	53	7.2
27	-	<5	-	-	2810	133	<5	-	-	224	-	56.5[c]	7.2
29	-	<5	260	170	2300	6	<5	<5	64	107	-	85[c]	7.6
36	NF[d]	NF	NF	NF	NF	610[e]	-	-	-	1580[e]	-	55	7.2
42	NF	NF	NF	NF	NF	520[e]	-	-	-	1460[e]	-	55	7.2

[a] Reactor TVSS equals 1400 mg/L during test.
[b] Blank means parameter not analyzed
[c] Temperature control malfunction.
[d] NF means not fed substrate at the time of sample collection.
[e] Data obtained after temperature shock.

maintained a mass loading of 0.04 mg phenol/mg TVSS/day throughout the test run.

Test data indicate relatively uniform treatment performance with the removal of cresol and acetic acid. However, variable effluent qualities for phenol and propionic acid may be the result of several system specific factors. The mass loading of phenol to the bioreactor could change the equilibrium concentration within the mixed liquor by no more than 50 mg/L for each day of operation. Therefore, an apparent rise in the mixed liquor phenol concentration of between 78 and 230 mg/L over a single day of bioreactor operation must be the result of factors other than the normal changes in biochemical reaction rate typical of microbial processes. One such condition is the accumulation of phenol within the flocs of sludge before actual biodegradation occurs. This accumulation probably results from sorption of phenol to the activated sludges because of the lipophilic properties of the flocs. Therefore, the biofloc may carry much higher concentrations of phenol than actually are measured in the liquid of the reactor. From time-to-time desorption may occur, liberating phenol and unknown metabolites to the reactor liquid. These compounds would be quickly taken up by the biomass and biodegraded in the normal cause of process operation. However, point-in-time measurements will show effluent quality variability as was reported by the test data. Bioadsorption of phenolics onto flocs is a phenomenon that was also observed with anaerobically treated sewage sludge (Giger et al. 1984), although the specific circumstances are not directly comparable because the research dealt with a biorefractory metabolite. The accumulation and eventual biodegradation of degradable pollutants on flocs is a common occurrence in activated sludge treatment. Whether this phenomenon presents problems specific to thermophilic anaerobic treatment of phenolics cannot be assessed from the experimental data.

During the performance testing period an equipment malfunction caused a rapid rise in bioreactor temperature which resulted in a complete kill of the microbial population. After the temperature control malfunction, the feed to

the reactors was discontinued. When the content of the bioreactor was sampled a week after the upset, an elevated concentration of phenol was found in the reactor liquid. This observation seems to verify the conclusion that bioaccumulation of phenolics is occurring during the treatment operation and that stabilization of the sludges (i.e., biodegradation of absorbed phenolics to organic acid intermediates) may require special considerations in process design.

Experimental data for a second performance test are presented in Table 11. This reactor was fed phenol at 1200 mg/L, p-cresol at 50 mg/L, and equal quantities of acetic and propionic acids (i.e., 250 to 350 mg/L). The bioreactor was operated with an hydraulic residence time of 20 days and a mean cell residence time of approximately 60 days. The system operated with a mass loading of 0.04 mg phenol/mg TVSS/day throughout the test run.

The effluent quality for this second performance test was similar to the first, although higher concentrations of phenol and COD appeared in the bioreactor liquid. The apparent variability in the effluent phenol quality is believed indicative of the accumulation and desorption phenomenon discussed previously. These data also indicate that proportional increases in reactor biomass relative to pollutant loading will not guarantee that the individual waste components will be uniformly degraded. Therefore, the apparent system kinetics may not fit a conventional microbial substrate utilization model.

5.3.2 Batch Biodegradation

A batch biodegradation study was conducted using the same acclimated thermophilic anaerobic microbes examined in the continuous-feed reactors. Experimental test data for the batch biodegradation study are presented in Table 12. This reactor was shock loaded to 100 mg/L of phenol, 50 mg/L of p-cresol, and 50 mg/L of both acetic and propionic acids. The test system was designed to observe the change in pollutant species with time and to identify

TABLE 11. PERFORMANCE TEST #2

Test Day	Influent					Effluent						Reactor[a]	
	Phenol (mg/L)	Cresol (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	COD (mg/L)	Phenol (mg/L)	Cresol (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	COD (mg/L)	TOC (mg/L)	Temp (°C)	pH
1	1170[b]	47	290	340	3530	6.7	<5	<5	75	190	145	53	7.1
4	-	-	320	370	3240	-	-	<5	-	-	-	-	-
5	1220	52	-	-	3510	20	<5	<5	66	-	-	55	7
6	-	-	-	-	-	-	-	5	-	-	-	54	7.05
7	-	-	-	-	-	24	<5	<5	76	-	-	54	7
8	-	46	360	480	3540	-	-	<5	-	190	-	53	7
11	1120	53	330	350	3430	<10	<5	15	-	-	-	53	7.1
12	-	-	350	300	-	360	<5	<5	75	160	134	53	7.1
13	-	-	290	350	3860	37	<5	10	90	86	100	53	7.05
14	-	-	280	300	-	112	<5	19	104	243	-	52.5	7.05
15	-	-	310	350	3620	-	<5	-	-	91	-	53	7.1
16	-	-	310	350	-	320	<5	4	31	560	-	-	-
17	-	-	280	320	-	-	<5	5	24	88	-	-	-
18	1240	31	270	300	3730	-	-	-	-	-	-	53	7.1
19	-	-	-	-	-	190	-	8	27	510	147	53	7.1
20	1220	69	260	260	3430	330	<5	<5	80	450	-	53	7.1
22	1300	39	270	280	3600	48	<5	9	29	100	118	53	7.1
25	-	41	280	360	3640	190	<5	<5	47	-	-	-	-
27	-	-	-	-	-	-	-	-	-	490	196	56.5	7.1
29	1220	39	270	360	3690	180	<5	6	90	490	104	85[c]	7.6
32	-	-	-	-	3670	-	-	-	-	-	-	-	-

[a] Reactor TVSS equals 1600 mg/L during test.
[b] Blank means parameter not analyzed.
[c] Temperature control malfunction.

TABLE 12. BATCH TREATMENT TEST[a]

Test Day	Phenol (mg/L)	Cresol (mg/L)	Acetic Acid (mg/L)	Propionic Acid (mg/L)	COD (mg/L)	TOC (mg/L)
1	104	50	40	30	-	-
3	108	49	<5	26	210	163
7	97	46	10	18	160	79
8	94	44	15	<5	250	102
9	115	49	<5	<5	170	-
10	93	<5	<5	<5	-	-
11	90	23	-	-	150	-
12	78	1.3	8	6	170	-
13	<5	<5	2	<5	150	121
15	<5	<5	<5	<5	180	-
16	<5	<5	-	-	150	124
17	<5	<5	-	-	-	-
21	<5	<5	<5	90	-	-
22	<5	<5	-	-	-	-

[a]Reactor TVSS equals 2000 mg/L during test.

the possible metabolic intermediates that characterized the thermophilic anaerobic degradations.

The experimental data indicate that the apparent removal rates for phenol and p-cresol were relatively slow during the first half of the test. However, these removal rates increased markedly in the second half, reducing the phenolics to below the analytical detection limit. This effect can be explained by the bioaccumulation theory, because the apparent phenolic levels in the reactor can be assumed to be residuals of prior waste additions that had not undergone biodegradation. However, as these degradations proceed, the available mass of substrate will decrease until the individual pollutants are reduced to negligible levels in the reactor. It would than follow, that at the point when the phenolics are at a minimum in the reactor liquid they are also at relatively low levels in the sludge. This would establish a zero level of sludge activity and place the biomass in a state of endogenous metabolism or decay. Operating under this condition would also serve to minimize the production of new cell mass.

In addition to the phenolics, several other compounds indicating possible phenol degradation intermediates were detected in several of the batch reactor effluent samples. The identity of these compounds cannot be definitely established with the information available. However, since the compounds appeared on the chromatograms prior to the phenol peak, it can be established that these compounds have boiling points lower than the boiling point of phenol (180°C). These unknown intermediates usually appeared just prior to a sharp increase in phenol concentration in the effluent and would usually carry through to the next sample collected 24 hours later. Coincident with the appearance of the peaks on the phenol chromatogram was the detection of butyric acid in the volatile organic acid analyzes. Butyric acid also appeared in the samples showing a sharp increase in phenol and would decrease in concentration in the samples collected 24 to 48 hours later. These results appear to be consistent with observations made by other investigators regarding degradation intermediates identified during mesophilic anaerobic treatment

of phenolics, which would indicate that the thermophilic process is similar to anaerobic treatment systems that operate at lower temperatures.

SECTION 6

REMAINING TECHNICAL QUESTIONS

6.1 INTRODUCTION

The feasibility of converting elevated concentrations of phenol to methane by thermophilic anaerobic biodegradation has been demonstrated by this study. However, a number of questions still remain concerning the practicality of using an anaerobic treatment process for phenolics removal as an alternative to a more conventional solvent extraction technology. These questions center on the following issues:

- The feasibility of also degrading to methane gas other phenolic compounds characteristic of fixed-bed gasification wastewaters,
- The feasibility of simultaneously degrading to methane gas a number of phenolic compounds in the presence of a wastewater pollutant matrix characteristic of most fixed-bed coal gasification quench condensates,
- The required pretreatments necessary to guarantee that thermophilic anaerobic biodegradation can proceed without microbial inhibition, and
- The data requirements for the development of kinetic formulations which adequately describe the biodegradations so that a practical design basis can be established. (This item is related to the technical area of identifying degradation intermediates which is a requirement for the formulation of biochemical and kinetic relationships typically used in an assessment of treatment performance.)

This section discusses the pollutant characteristics of fixed-bed gasifier quench condensates and how the treatment of these wastewaters might be studied in order to assess the technical feasibility of applying thermophilic anaerobic treatment in lieu of solvent extraction for phenolics removal.

6.2 TYPICAL FIXED-BED GASIFICATION QUENCH CONDENSATE CHARACTERISTICS

Quench condensate is the principal wastewater produced from coal gasification facilities that use wet scrubbing of raw product gases. This stream is important in wastewater treatment design because it represents the largest and most contaminated aqueous stream from gasification processes. Quench condensates from fixed-bed processes contain several different categories of pollutants. These are dissolved inorganic gases, dissolved organics, suspended solids, and dissolved inorganics. The complex composition of quench condensates dictates that multiple treatments are utilized to improve the water quality.

The wastewater quality of a typical fixed-bed gasification quench condensate is presented in Table 13. These data are for a Lurgi-type fixed-bed coal gasification process that produces a medium-BTU gas from the gasification of lignite (Lee et al. 1981). The analysis of the quench condensate indicates that of the identified compounds, the phenolics account for the largest percentage of the total chromatographable organics. Methylphenols contribute the majority of the phenolics identified, with phenol the next largest contributor. Lurgi-type process quench condensates also contain such trace elements as arsenic, calcium, chromium, iron, magnesium, phosphorus, potassium, silicon, sodium, and sulfur (Lee et al. 1981).

6.3 PRETREATMENT REQUIREMENTS FOR ANAEROBIC BIODEGRADATION

The treatment of fixed-bed gasification quench condensates by anaerobic biodegradation would first need to consider pretreatments to reduce ammonia and acid gases to within acceptable concentrations to prevent upset of a biological treatment process. One approach to pretreatment is indicated by the process schematic presented in Figure 4. This pretreatment system is designed to reduce the concentration of ammonia and acid gases in the wastewater before the wastewater is introduced into the anaerobic reactor. Lime coagulation is used to raise the pH of the wastewater prior to

TABLE 13. CHARACTERIZATION OF TYPICAL LURGI-TYPE QUENCH CONDENSATE

Parameter[a]	Gas Liquor Quality[b]
pH, standard units	9.2
Chemical Oxygen Demand	18,900
Biochemical Oxygen Demand	9,030
Total Organic Carbon	4,970
Total Dissolved Solids	2,170
Total Suspended Solids	150
Free Ammonia	3,510
Thiocyanate	<75
Phenol	690
2,4-Dimethylphenol	130
Dimethylphenols (total)[c]	333
2-Methylphenol (o-Cresol)	260
3-Methylphenol (m-Cresol)	610
4-Methylphenol (p-Cresol)	100
Unknown Phenolics	698
Pyridine	28
Ethylpyridine(s)	46
2-Methylpyridine	29
Alkylpyridine(s)	26

[a]All values as mg/L except where noted.
[b]Lurgi-type medium-BTU gasifier at KOSOVO, Yugoslavia (Lee et al. 1981).
[c]Includes 2,4-Dimethylphenol.

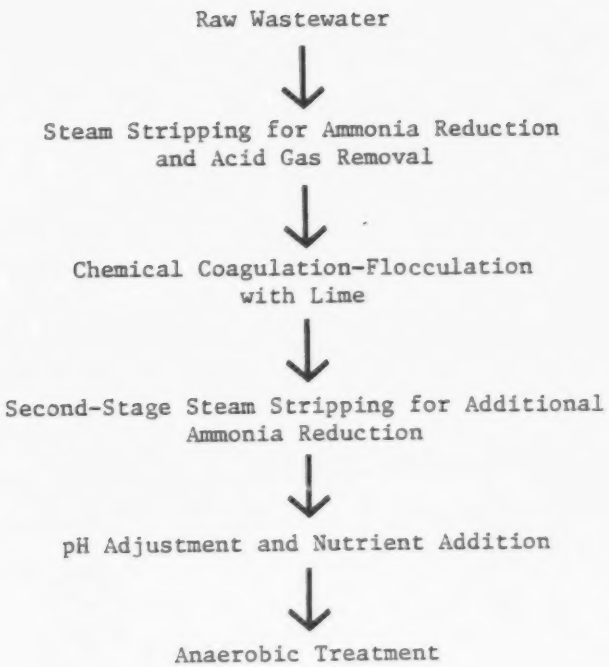

Raw Wastewater
Steam Stripping for Ammonia Reduction and Acid Gas Removal
Chemical Coagulation-Flocculation with Lime
Second-Stage Steam Stripping for Additional Ammonia Reduction
pH Adjustment and Nutrient Addition
Anaerobic Treatment

Figure 4. Pretreatment considerations for anaerobic biodegradation

second-stage steam stripping because this should reduce the concentration of dissolved solids in the quench condensate. An ammonia level of less than 500 mg/L could be the preliminary target value for an experimental design. However, pretreated wastewater residual alkalinity concentrations will be the controlling factor because of the need to maintain sufficient buffer capacity in the waste to stabilize pH during volatile acid production in the anaerobic reactor.

Target levels for free cyanide and sulfide are less than 0.05 mg/L and 50 mg/L, respectively. The quantity of phenol and other phenolic compounds in the quench condensates are loaded to the bioreactors as received. During wastewater acclimation the wastes are step-fed to reactors, that have been seeded with combinations of previously acclimated sludges, by slowly increasing phenol concentration (pollutant strength) over a finite period of operation. The feasibility of the anaerobic treatment process for phenolic removal from quench condensates is determined by how well the bioreactor responds to the treatment of a complex wastewater matrix. Therefore, experimentation with actual fixed-bed coal gasification quench condensates would be required before the feasibility of this treatment process can be established.

6.4 TECHNICAL QUESTIONS

In order to establish a data base for process evaluation, the following information should be obtained:

- The conditions for acclimation of seed bacteria with the capacity to perform the anaerobic decomposition of a variety of phenolic compounds in addition to simple phenol (i.e., p-cresol, o-cresol, and other substituted phenols).
- Factors that establish optimal environmental conditions for the growth of acetogenic and methanogenic bacteria in a thermophilic temperature range.
- Identification of key metabolic intermediates and use these compounds to better understand the biokinetics of the process.

- Development of an energy balance on the process in order to establish whether the process has a favorable energy yield.

- Development of kinetic and performance data for the process using actual coal gasification wastewaters from candidate demonstration- or commercial-scale gasifiers.

- Confirmation of process kinetics using wastewaters representative of several coal-types.

Compounds that cannot be anaerobically biodegraded but which do not produce an apparent biotoxicity effect may be biodegradable under aerobic conditions and, as such, a modification of this treatment could be evaluated which employs a combined anaerobic/aerobic biooxidation system. Compounds which represent severe toxicity problems for the thermophilic anaerobic treatment process and are not biodegradable even at low concentrations, could be examined in order to define possible pretreatments that might minimize their toxicity to the process. Such pretreatments should be assessed in terms of their relative impact on treatment process economics.

If anaerobic treatment is to be practicable, it should be accomplished at phenolic levels close to the maximum concentrations which characterize fixed-bed coal gasification wastewaters. A given feasibility study should emphasize an approach which strives to achieve optimum phenolic loadings on the anaerobic system by taking into account the characteristic concentration levels of all other wastewater constituents (e.g., thiocyanate, ammonia) relative to the minimum pretreatment requirements established for the individual pollutants. This would allow a more realistic evaluation of toxic effects.

SECTION 7

REFERENCES

Blum, D.J.W., R. Hergenroeder, G. F. Parkin, and R.E. Speece (1984), "Anaerobic Treatment of Coal Conversion Wastewater Constituents: Biodegradability and Toxicity", Presented at the 57th Water Pollution Control Federation Conference, New Orleans, Louisiana.

Bryant, M.P., Wolin, E.A., and R.S. Wolf (1967) "Methanobacillus Omelianskii, A symbiotic Association of Two Species of Bacteria", Archiv. fur Mikeobiologic, p.20.

Chmielowski, J., A. Grossman and S. Labuzck (1965) "Biochemical Degradation of Some Phenols During Methane Fermentation", Zesz. Nauk. Politech. Slasha, Inz. Sanit., No. 8, p. 97.

Chmielowski, J. and W. Wasilewski (1966) "A Investigation of the Dynamics of Anaerobic Decomposition of Some Phenols in Methane Fermentation", Zesz. Nauk. Politech. Slasha, Inz. Sanit., No. 9, p. 95.

Clark, F.M. and L.R. Fina (1952) "The Anaerobic Decomposition of Benzoic Acid During Methane Fermentation", Archives of Biochemistry and Biophysics, Vol. 36, p. 26.

Eckenfelder, W.W. (1966) Industrial Water Pollution Control, McGraw-Hill Book Company, New York, 275 pp.

Fedorak, P.M. and S.E. Hrudey (1984) "The Effects of Phenol and Some Alkyl Phenolics on Batch Anaerobic Methanogenesis", Water Research, Vol. 18, No. 3, p. 361.

Gallagher, J.R. (1984) University of North Dakota, personal communication.

Giger, W., P.H. Brunner, and C. Schaffner (1984), "4-Nonylphenol in Sewage Sludge: Accumulation of Toxic Metabolites from Nonionic Surfactants", Science, Vol. 225, p. 623.

Healy, J.B. and L.Y. Young (1979) "Anaerobic Biodegradation of Eleven Aromatic Compounds to Methane", Applied and Environmental Microbiology, Vol. 38, No. 1, p. 84.

Kaiser, J.P. and K.W. Hanselmann (1982) "Fermentative Metabolism of Substituted Monoaromatic Compounds by a Bacterial Community from Anaerobic Sediments", Archives of Microbiology, Vol. 133, p. 185.

Keith, C.L. (1973) "Intermediates in the Anaerobic Metabolism of Benzoic Acid", Dissertation Abstracts, Vol. 33, No. 7.

Khan, K.A., Suidan, M.T. and W.H. Cross (1981) "Anaerobic Activated Carbon Filter for the Treatment of Phenol-Bearing Wastewater:, Journal Water Pollution Control Federation, Vol. 53, No. 10, p. 1519.

Lawrence, A.W. and P.L. McCarty (1969) "Kinetics of Methane Fermentation in Anaerobic Treatment", Journal Water Pollution Control Federation, Vol. 41, No. 2, pt.2, pR1.

Lee, K.W., W.S. Seames, R.V. Collins, K.J. Bombaugh, and G.C. Page (1981), "Environmental Assessment: Source Test and Evaluation Report -- Lurgi (KOSOVO) Medium-BTU Gasification", PB82-114075, EPA 600/7-81-142.

Neufeld, R.D., J.D. Mack, and J.P. Strakey (1980) "Anaerobic Phenol Biokinetics" Journal Water Pollution Control Federation, Vol. 52, No. 9, p. 2367.

Parkin, G.F. and R.E. Speece (1984) "Anaerobic Biological Waste Treatment", Chemical Engineering Progress, p. 55.

Smith, P.H. (1980) "Studies of Methanogenic Bacteria In Sludge", EPA-600/2-80-093, 99 pp.

Suidan, M.T., C.E. Strubler, S.W. Kao, and J.T. Pfeffer (1982) "Treatment of Coal Gasification Wastewater With Anaerobic Filter Technology", Presented 55th Water Pollution Control Federation conference, St. Louis, Missouri.

Switzenbaum, M.S. (1983) "Anaerobic Treatment of Wastewater: Recent Developments" The American Society for Microbiology (News), Vol. 49, No. 11, p. 532.

Wang, Y.T., M.T. Suidan, and J.T. Pfeffer (1984) "Anaerobic Biodegradation of Indole to Methane", Applied and Environmental Microbiology, Vol. 48, No. 5, p. 1058.

Williams, R.J. and W.C. Evans (1973) "Anaerobic Metabolism of Aromatic Substrates by Microorganisms", Biochemical Soc. Trans., Vol. 1, p. 186.

APPENDIX

ANALYTICAL QUALITY CONTROL DATA

DUPLICATE SAMPLE ANALYSIS - PHENOL

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG.	% DIFFERENCE
E1-06-20	323	315	319	
E2-06-20	335	323	329	3.7
E1-06-27	339	323	331	5.0
E2-06-27	339	333	336	1.8
E1-07-20	36	35	36	2.9
E2-07-20	144	138	141	4.3
E1-07-23	138	137	138	0.7
E2-07-23	160	148	154	8.1
E1-07-20 redo	83	79	81	5.1
E2-07-20 redo	192	188	190	2.1
E1-07-25	95	85	90	11.8
E2-07-25	320	316	318	1.3
E1-07-27	121	109	115	11.0
E2-07-27	300	283	292	6.0
E1-07-30	146	129	138	13.2
E2-07-30	340	308	324	10.4
E1-08-01	109	106	108	2.8
E2-08-01	264	258	261	2.3
EI-08-03	71	54	63	31.5
E2-08-03	253	228	241	11.0
E1-08-06	87	82	85	6.1
E2-08-06	238	237	238	0.4
E1-08-08	115	109	112	5.5
E2-08-08	194	179	187	8.4
E1-08-10	114	88	101	29.5
E2-08-10	167	162	165	3.1
E1-08-13	134	69	102	94.2
E2-08-13	134	124	129	8.1
E1-08-16	50	50	50	0.0
E2-08-16	143	136	140	5.1
E1-08-17	48	48	48	0.0
E2-08-17	138	130	134	6.2
E1-08-22	84	81	83	3.7
E2-08-22	174	159	167	9.4
E1-08-27	56	49	53	14.3
E2-08-27	159	157	158	1.3
E1-08-29	47	46	47	2.2
E2-08-29	176	176	176	0.0

DUPLICATE SAMPLE ANALYSIS - PHENOL

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG.	% DIFFERENCE
E1-08-31	21	16	19	31.3
E2-08-31	180	174	177	3.4
E1-09-12	3.9	3.7	4	5.4
E1-09-14	3.9	3.9	4	0.0
E3-09-14	<1	<1	0	0.0
E1-09-17	13	12	13	8.3
E1-09-19	27	26	27	3.8
E3-09-19	<1	<1	0	0.0
I3-09-24	78	71	75	9.9
E1-09-28	4.8	4.7	5	2.1
E1-10-01	5.4	5.2	5	3.8
E2-10-01	22	19	21	15.8
E2-10-03	5.4	4.4	5	22.7
E2-10-08	23	21	22	9.5
E6-12-15	<5	<5	<5	0.0
E6-12-14	<5	<5	<5	0.0
E7-12-21	49	45	47	8.9
E7-12-11	390	330	360	18.2
E6-12-18	123	110	117	11.8
E6-12-26	146	133	140	9.8
E6-12-09	288	240	264	20.0

a All results as mg/L.

b % difference = $\frac{[\text{ result 1 - result 2 }]* 100}{\text{result 2}}$

DUPLICATE SAMPLE ANALYSIS - COD

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG	% DIFFERENCE
I-05-25	1820	1725	1773	5.5
E1-05-25	682	621	652	9.8
E2-05-25	510	490	500	4.1
I-05-31	1020	1020	1020	0.0
E1-05-31	595	583	589	2.1
E2-05-31	707	482	595	46.7
I-06-02	1860	1830	1845	1.6
E1-05-31 redo	924	906	915	2.0
E2-05-31 redo	834	828	831	0.7
I-06-07	1682	1624	1653	3.6
E1-06-07	882	882	882	0.0
E2-06-07	986	893	940	10.4
I-06-13	1232	1232	1232	0.0
E1-06-13	504	504	504	0.0
E2-06-13	347	347	347	0.0
I-06-18	4172	4116	4144	1.4
E1-06-18	644	633	639	1.7
E2-06-18	476	442	459	7.7
I-06-20	3699	3645	3672	1.5
E1-06-20	967	956	962	1.2
E1-06-20	1010	967	989	4.4
I-06-27	1204	1204	1204	0.0
E1-06-27	1092	1092	1092	0.0
E2-06-27	1036	1036	1036	0.0
I-07-03	1742	1378	1560	26.4
E1-07-03	910	858	884	6.1
E2-07-03	1014	910	962	11.4
I-07-11	2093	2046	2070	2.3
E1-07-11	558	465	512	20.0
E2-07-11	1209	1209	1209	0.0
I-07-16	1840	1748	1794	5.3
E1-07-16	552	552	552	0.0
E2-07-16	920	644	782	42.9
I-07-18	1978	1932	1955	2.4
E1-07-18	414	345	380	20.0
E2-07-18	874	759	817	15.2
I-07-20	2070	2024	2047	2.3
E1-07-20	589	580	585	1.6
E2-07-20	819	796	808	2.9
I-07-23	1978	1932	1955	2.4
E1-07-23	552	542	547	1.8
E2-07-23	846	837	842	1.1

DUPLICATE SAMPLE ANALYSIS - COD

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG	% DIFFERENCE
I-07-25	2050	1900	1975	7.9
E1-07-25	370	360	365	2.8
E2-07-25	780	740	760	5.4
I-07-27	2500	2050	2275	22.0
E1-07-27	510	500	505	2.0
E2-07-27	1040	960	1000	8.3
I-07-30	1775	1675	1725	6.0
E1-07-30	505	475	490	6.3
E2-07-30	1010	870	940	16.1
I-08-01	1675	1575	1625	6.3
E1-08-01	435	375	405	16.0
E2-08-01	870	750	810	16.0
I-08-03	1725	1625	1675	6.2
E1-08-03	355	335	345	6.0
E2-08-03	730	670	700	9.0
I-08-06	2184	2184	2184	0.0
E1-08-06	510	510	510	0.0
E2-08-06	749	686	718	9.2
I-08-08	1475	1425	1450	3.5
E1-08-08	665	565	615	17.7
E2-08-06	790	690	740	14.5
I-08-10	2107	1911	2009	10.3
E1-08-10	578	578	578	0.0
E2-08-10	902	882	892	2.3
I-08-15	2890	2198	2544	31.5
E1-08-15	272	232	252	17.2
E2-08-15	425	405	415	4.9
E2-08-17	545	537	541	1.5
I-08-20	1517	1498	1508	1.3
AE-09-19	228	210	219	8.6
I1-11-14	3456	3360	3408	2.9
I4-11-14	1056	960	1008	10.0
I5-11-14	960	672	816	42.9
E1-11-14	250	250	250	0.0
E4-11-14	547	518	533	5.6
E5-11-14	1753	1434	1594	22.2
I1-11-19	3300	3100	3200	6.5
I4-11-19	800	780	790	2.6
I5-11-19	240	240	240	0.0
E1-11-19	370	360	365	2.8
E4-11-19	700	610	655	14.8
E5-11-19	2350	1250	1800	88.0

DUPLICATE SAMPLE ANALYSIS - COD

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG	% DIFFERENCE
I1-11-16	2976	2880	2928	3.3
I4-11-16	576	480	528	20.0
I5-11-16	96	48	72	100.0
E1-11-16	355	346	351	2.6
E4-11-16	624	586	605	6.5
E5-11-16	1152	1152	1152	0.0
I1-11-21	3696	2928	3312	26.2
I4-11-21	893	874	884	2.2
I5-11-21	533	494	514	7.9
E1-11-21	322	283	303	13.8
E4-11-21	590	581	586	1.5
E5-11-21	778	778	778	0.0
I1-11-26	2790	2666	2728	4.7
I4-11-26	682	682	682	0.0
E1-11-26	428	415	422	3.1
E4-11-26	787	763	775	3.1
I4-11-28	1004	992	998	1.2
E4-11-28	546	496	521	10.1
I4-11-30	1643	1519	1581	8.2
I6-11-30	3038	3038	3038	0.0
I7-11-30	3534	3534	3534	0.0
E4-11-30	651	589	620	10.5
E6-11-30	583	533	558	9.4
E7-11-30	390	378	384	3.2
E8-11-30	266	229	248	16.2
I4-12-03	999	945	972	5.7
I6-12-03	3308	3038	3173	8.9
I7-12-03	3186	3294	3240	-3.3
E4-12-03	616	594	605	3.7
E6-12-03	421	421	421	0.0
E7-12-03	659	637	648	3.5
E8-12-03	275	265	270	3.8
I4-12-05	1350	1242	1296	8.7
I6-12-05	3072	2862	2967	7.3
I7-12-05	3510	3510	3510	0.0
E4-12-05	637	637	637	0.0
E6-12-05	637	637	637	0.0
E7-12-05	1026	983	1005	4.4
I4-12-07	1186	1165	1176	1.8
I6-12-07	3120	3120	3120	0.0
I7-12-07	3640	3432	3536	6.1
E4-12-07	562	562	562	0.0

DUPLICATE SAMPLE ANALYSIS - COD

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG	% DIFFERENCE
E6-12-07	270	270	270	0.0
E7-12-07	520	520	520	0.0
I4-12-10	1269	1269	1269	0.0
I6-12-10	2600	2288	2444	13.6
I7-12-10	3432	3432	3432	0.0
E4-12-10	437	426	432	2.6
E6-12-10	354	354	354	0.0
E7-12-10	1248	1227	1238	1.7
I4-12-12	1350	1350	1350	0.0
I6-12-12	3170	3120	3145	1.6
I7-12-12	4060	3660	3860	10.9
E4-12-12	380	360	370	5.6
E6-12-12	430	410	420	4.9
I4-12-14	1361	1361	1361	0.0
I6-12-14	2916	2862	2889	1.9
I7-12-14	3672	3564	3618	3.0
E4-12-14	486	486	486	0.0
E6-12-14	994	929	962	7.0
E7-12-14	1274	1253	1264	1.7
I4-12-17	1123	1080	1102	4.0
I6-12-17	2592	2538	2565	2.1
I7-12-17	3780	3672	3726	2.9
E4-12-17	346	335	341	3.3
E6-12-17	475	454	465	4.6
E7-12-17	670	605	638	10.7
I4-12-19	1622	1622	1622	0.0
I6-12-19	2964	2964	2964	0.0
I7-12-19	3432	3432	3432	0.0
E4-12-19	322	312	317	3.2
E6-12-19	946	946	946	0.0
E7-12-19	1456	1456	1456	0.0
I4-12-21	1722	1722	1722	0.0
I6-12-21	2730	2624	2677	4.0
I7-12-21	3657	3551	3604	3.0
E4-12-21	313	313	313	0.0
E6-12-21	604	562	583	7.5
E7-12-21	625	562	594	11.2
I4-12-26	2093	1988	2041	5.3
I6-12-26	2889	2836	2863	1.9
I7-12-26	3737	3684	3711	1.4
E4-12-26	429	419	424	2.4
E6-12-26	906	896	901	1.1
E7-12-26	588	567	578	3.7

DUPLICATE SAMPLE ANALYSIS - COD

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG	% DIFFERENCE
I4-12-28	2184	2132	2158	2.4
I6-12-28	2340	2236	2288	4.7
I7-12-28	3744	3640	3692	2.9
E4-12-28	270	218	244	23.9
E6-12-28	998	998	998	0.0
E7-12-28	801	790	796	1.4
I4-12-31	1466	1466	1466	0.0
I6-12-31	1550	1529	1540	1.4
I7-12-31	3666	3666	3666	0.0
E4-12-31	213	192	203	10.9
E6-12-31	130	130	130	0.0
E7-12-31	2002	2002	2002	0.0

a All results as mg/L.

b % difference = $\frac{[\text{result 1} - \text{result 2}] * 100}{\text{result 2}}$

DUPLICATE SAMPLE ANALYSIS - ACETIC ACID

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG.	% DIFFERENCE
E7-11-30	<5	<5	<5	0
E7-12-05	10	5	8	100
E7-12-13	7	6	6	17
E7-12-15	<5	4	<5	-
E6-12-06	<5	<5	<5	0
E6-12-09	6	<5	6	-
E6-12-13	5	5	5	0
E6-12-16	10	6	8	67
E6-12-18	9	8	9	13
E6-12-21	<5	<5	<5	0
I6-11-30	244	239	242	2
I6-12-14	96	94	95	2
I6-12-21	<5	<5	<5	0
I7-11-30	294	274	284	7
I7-12-07	372	343	358	8
I7-12-13	282	277	280	2
E8-12-09	<5	<5	<5	0
E8-12-13	7	<5	7	-
I7-12-04	330	310	320	6
I6-12-10	230	220	225	5

a All results as mg/L.

b % difference $= \frac{(\text{result 1} - \text{result 2}) * 100}{\text{result 2}}$

DUPLICATE SAMPLE ANALYSIS - PROPIONIC ACID

SAMPLE IDENTIFICATION	RESULT #1	RESULT #2	AVG.	% DIFFERENCE
E7-12-05	18	<5	-	-
E7-12-13	33	25	29	32
E7-12-15	<5	<5	<5	0
E6-12-06	<5	<5	<5	0
E6-12-13	12	11	12	9
E6-12-18	16	<5	-	-
E6-12-21	120	93	107	29
I6-11-30	297	290	294	2
I6-12-14	115	113	114	2
I7-11-30	360	320	340	13
I7-12-07	272	256	264	6
I7-12-13	310	305	308	2
E8-12-09	<5	<5	<5	0
E8-12-13	<5	<5	<5	0
I7-12-04	400	370	385	8
I6-12-10	230	200	215	15

a All results as mg/L.

b % difference $= \frac{[\text{ result 1} - \text{result 2 }] * 100}{\text{result 2}}$